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A new species of *Gracixalus* (Anura, Rhacophoridae) from Guangxi, China, with comments on the taxonomy of *Orixalus*

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Abstract

A new treefrog of the genus *Gracixalus*, *Gracixalus huaping* **sp. nov.**, is described based on the specimens collected from Huaping National Natural Reserve, Guangxi, China. Both maximum likelihood analysis and Bayesian inference found that the Huaping population formed a distinct lineage as sister to *Gracixalus jinxiuensis*. It was strongly supported that *Gracixalus* consists of four distinct clades. Morphological analysis suggested that the new species can be distinguished from other known congeners by a combination of the following morphological characters: size small, SVL 26.6–28.8 mm in males and 29.8–32.8 mm in females; vomerine teeth absent; dorsal surface yellowish brown; internal vocal sac and linea masculina present in males; vocal sac opening oval and rim of the opening dark brown; throat smooth; iris golden brown; flanks granular with white blotch. In addition, we agree that the genus *Orixalus* is a junior subjective synonym of *Gracixalus* and consider that more studies are needed to investigate the species diversity and taxonomy of genus *Gracixalus*.

Key Words

Gracixalus jinxiuensis, species diversity, taxonomy, Treefrog

Introduction

The genus *Gracixalus* sensu lato Delorme, Dubois, Grosjean & Ohler, 2005, a group comprised of small to medium-sized (SVL 20.0–41.6 mm) rhacophorids (Boruah et al. 2023), was erected by Delorme et al. (2005) and currently is known from Thailand, Myanmar, Laos, Vietnam, India, and southern and southwestern China (Frost 2024). It was originally coined as a subgenus of *Aquixalus* Delorme, Dubois, Grosjean & Ohler, 2005, and was later raised to the rank of genus with *Aquixalus* sensu stricto being transferred to *Kurixalus* Ye, Fei & Dubois, 1999 (Li et al. 2008; Yu et al. 2009). Recently, based on a phylogeny containing eight species of *Gracixalus* sensu lato, Dubois et al. (2021) split *Gracixalus* sensu lato into *Orixalus* Dubois, Ohler & Pyron, 2021, and *Gracixalus* sensu stricto as sister genera; the former contains *G. ananjevae* (Matsui & Orlov, 2004), *G. carinensis* (Boulenger, 1893), *G. jinxiuensis* (Hu, 1978), and *G. nonggangensis* Mo, Zhang, Luo, Zhou & Chen, 2013, and the latter contains other species. Subsequently, this taxonomic change was adopted by the classification of Frost (2024), in which *Gracixalus* sensu stricto contains 16 species and *Orixalus* is composed of the four species mentioned above. However, according to recent molecular phylogenetic analyses based on wide sampling (Chen et al. 2018; Yu et al. 2019; Tran et al. 2023; Boruah et al. 2023), *Orixalus* and *Gracixalus* sensu stricto are not monophyletic to each other, indicating that the generic status of *Orixalus* is problematic. Moreover, Mahony et al. (2024) argued that nominal *Orixalus* is not morphologically diagnosable from *Gracixalus* and therefore considered *Orixalus* a junior subjective synonym of *Gracixalus*.

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Thus, following the classification of Mahony et al. (2024), the genus *Gracixalus* currently contains 20 species globally (the 16 species placed in *Gracixalus* sensu stricto plus the four species of *Orixalus*; see Frost 2024), and more than half of them (13 species) were described from southern China and Vietnam in the last decade, indicating that species diversity of this genus has been heavily underestimated. To date, a total of nine species of the genus *Gracixalus* have been recognized in China, and four of them are distributed in Guangxi, namely *G. gracilipes* (Bourret, 1937), *G. jinxiuensis*, *G. nonggangensis*, and *G. tianlinensis* Chen, Bei, Liao, Zhou & Mo, 2018 (AmphibiaChina 2024). Guangxi is a part of the Indo-Burma Biodiversity Hotspot and is second only to Yunnan in amphibian species diversity in China (Tang et al. 2023). In recent years, a series of new amphibian species were discovered in Guangxi (e.g., Lyu et al. 2019, 2021; Li et al. 2022; Chen et al. 2022a, 2022b), indicating that amphibian species diversity of Guangxi requires further investigation. *Gracixalus jinxiuensis* was once recorded widely from Guangxi, Hunan, Yunnan, Vietnam, and Laos, but Yu et al. (2019) revealed that previous records of this species from Yunnan, Vietnam, and Laos actually apply to *G. yunnanensis* Yu, Li, Wang, Rao, Wu & Yang, 2019, and the record of this species from Hunan belongs to *G. guangdongensis* Wang, Zeng, Liu & Wang, 2018.

During fieldwork in northeastern Guangxi, China, we collected some specimens of a small-sized tree frog resembling *G. jinxiuensis* from Huaping National Natural Reserve, Longsheng County. Phylogenetic analyses recovered these specimens as a distinct lineage of genus *Gracixalus*, and morphological examination demonstrated that this lineage is distinguished from known members of the genus *Gracixalus* by a series of characters. Herein, we officially describe this lineage as a new species of *Gracixalus*.

Materials and methods

Sampling

Specimens were collected from Huaping National Natural Reserve, Longsheng, Guangxi, China, by Guohua Yu in May 2022 (Fig. 1). The specimens were euthanized with ethyl acetate, fixed, and then stored in 75% ethanol. Liver tissues were preserved in 99% ethanol. All specimens were deposited at Guangxi Normal University (**GXNU**; Table 1).

Phylogenetic analyses and species delimitation

Total genomic DNA was extracted from liver tissues. Tissue samples were digested using proteinase K and subsequently purified following a standard phenol/chloroform

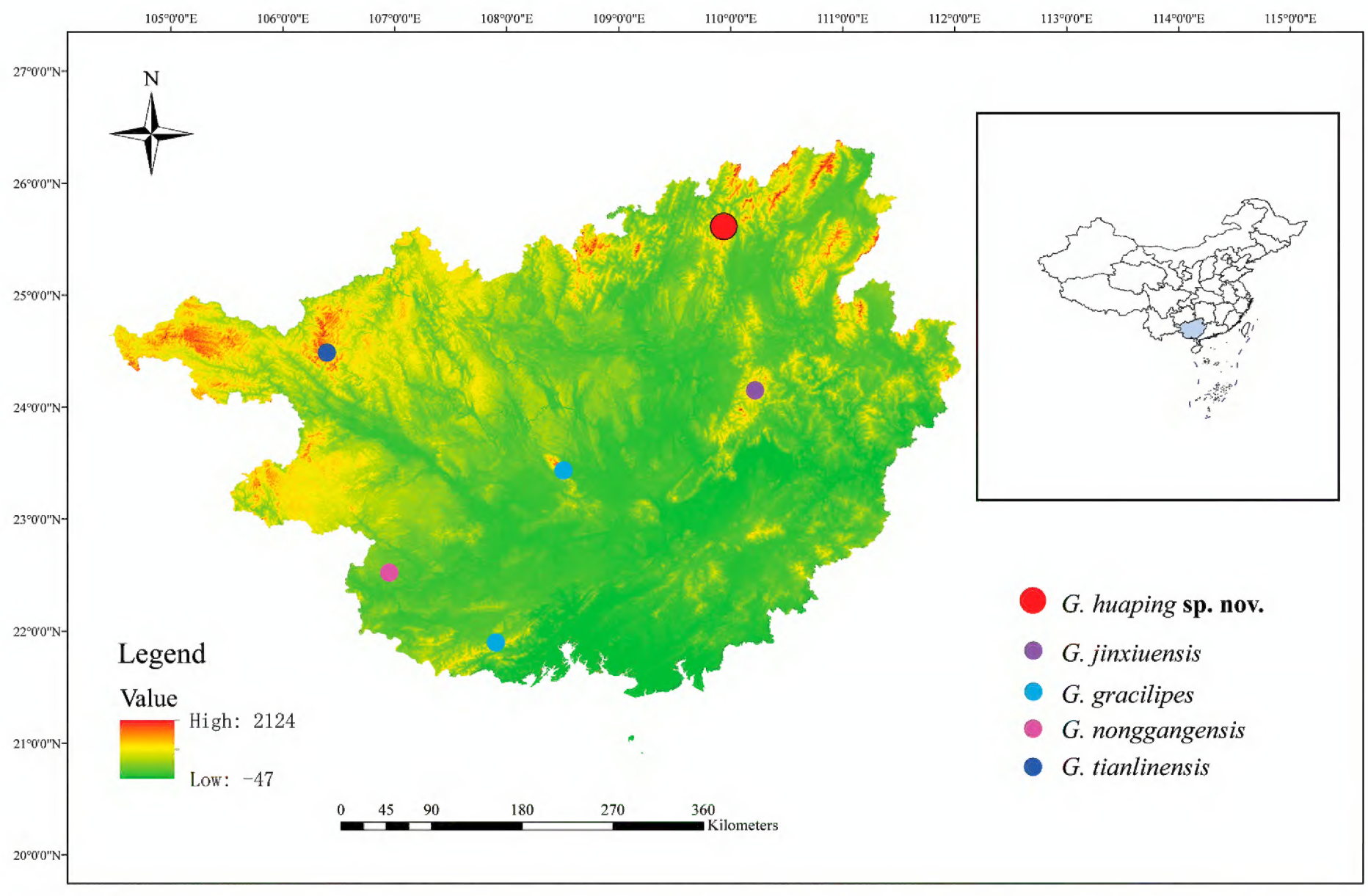

Figure 1. The distribution of *Gracixalus* species in Guangxi, China. The red circle represents the type locality of the new species (Huaping, Longsheng). The map was generated using ArcMap v.10.7.

isolation and ethanol precipitation. A fragment encoding a partial 16S rRNA gene was amplified and sequenced following Yu et al. (2010). All new sequences have been deposited in GenBank under Accession Nos. PP083976–PP083980 (Table 1). Forty-nine homologous sequences from 19 species of *Gracixalus* were obtained from GenBank. *Rhacophorus borneensis* Matsui, Shimada & Sudin, 2013, and *Kurixalus idiootocus* (Kuramoto & Wang, 1987) were selected as outgroups according to Matsui et al. (2017), and sequences of them were also downloaded from GenBank.

Sequences were aligned using MUSCLE with the default parameters in MEGA version 11 (Tamura et al. 2021). Uncorrected pairwise distances between species were calculated in MEGA version 11. The best substitution model (GTR + I + G) was selected using the corrected Akaike Information Criterion (AICc) in jMODELTEST version 2.1.10 (Darriba et al. 2012). Two methods were used to construct the phylogeny of the genus *Gracixalus*. Firstly, maximum likelihood analysis was performed in RaxmlGUI version 2.0 (Edler et al. 2021) with 1000 rapid bootstrap replicates. Secondly, Bayesian inference was conducted in MrBayes version 3.2.6 (Ronquist et al. 2012) based on the selected substitution model. Two runs were performed simultaneously with four Markov chains starting from a random tree. The chains were run for 5,000,000 generations and sampled every 100 generations. Convergence and burn-in were checked using the program Tracer version 1.6. (Rambaut et al. 2014). The first 25% of the sampled trees were discarded as burn-in, and the remaining trees were used to create a consensus tree and to estimate Bayesian posterior probabilities (BPPs).

Species delimitation was conducted using the method of Assemble Species by Automatic Partitioning (ASAP; Puillandre et al. 2020) and the substitution model of simple distance (*p*-distances). We chose the partitioning with the lowest ASAP score as the best according to Puillandre et al. (2020). In addition, to delimit species boundaries, the method of Bayesian Poisson Tree Processes (bPTP; Zhang et al. 2013) was run on the bPTP server (http://species.h-its.org/) based on the Bayesian phylogenetic tree and default parameters.

Morphology

Morphometric data were taken using electronic digital calipers to the nearest 0.1 mm. The morphological terminologies followed Yu et al. (2019). A total of 13 characters were measured, including snout-vent length (SVL), head length (HL), head width (HW), snout length (SL), internarial distance (IND), interorbital distance (IOD), eye diameter (ED), upper eyelid width (UEW), tympanum diameter (TD), forearm and hand length (FHL), tibia length (TL), length of tarsus and foot (TFL), and foot length (FL). The principal component analysis (PCA) was performed using R version 4.4.0 with the FactoMineR package (Lê et al. 2008), based on the correlation matrix of size-standardized measurements (all measurements divided by SVL), and the factoextra and ggplot2 (Wickham 2016) packages were used to extract and visualize the results. We used the scatter plots of the first two PCA factors to examine the differentiation between the new species and its closest relative (*G. jinxiuensis*) revealed by phylogenetic analyses.

Comparative morphological data of other species of *Gracixalus* were taken from their original descriptions or re-descriptions (Boulenger 1893; Hu et al. 1978; Ye and Hu 1984; Orlov et al. 2004; Matsui and Orlov 2004; Nguyen et al. 2008, 2013; Rowley et al. 2011, 2014, 2020; Mo et al. 2013; Matsui et al. 2015, 2017; Zeng et al. 2017; Chen et al. 2018; Wang et al. 2018; Yu et al. 2019; Le et al. 2021; Tran et al. 2023; Boruah et al. 2023). Webbing formula followed Myers and Duellman (1982).

Results

Phylogenetic relationship and species delimitation

After trimming both ragged sides, the obtained alignment of 16S rRNA sequences is 547 bp in length. Both maximum likelihood analysis and Bayesian inference recovered the Huaping population as a distinct lineage and as sister to *G. jinxiuensis* (Fig. 2). It was strongly supported that *Gracixalus* consists of four distinct clades: one containing *G. lumarius* Rowley, Le, Dau, Hoang & Cao, 2014 (clade I); one containing *G. seesom* Matsui, Khonsue, Panha & Eto, 2015, *G. quyeti* (Nguyen, Hendrix, Böhme, Vu & Ziegler, 2008), *G. quangi* Rowley, Dau, Nguyen, Cao & Nguyen, 2011, *G. supercornutus* (Orlov, Ho & Nguyen, 2004), *G. gracilipes*; and *G. patkaiensis* Boruah, Deepak, Patel, Jithin, Yomcha & Das, 2023 (clade II); one containing *G. medogensis* (Ye & Hu, 1984) (clade IV); and one containing other members and the Huaping population (clade III). The average uncorrected pairwise distances (*p*-distances) between this novel lineage and known species of *Gracixalus* ranged from 3.0% (*G. jinxiuensis*) to 15.2% (*G. lumarius*) (Table 2).

The best partition (score = 3.0) revealed by the ASAP analysis assigned the samples of genus *Gracixalus* used in this study into 25 putative species, and one of them corresponds to the lineage comprised of the Huaping population. All other samples were assigned into corresponding species with the exceptions of *G. nonggangensis* and *G. guangdongensis*, both of which were assigned into three putative species (Fig. 3). Being consistent with the ASAP analysis, the bPTP analysis also supported the lineage containing specimens from Huaping as a putative species, but it split *G. guangdongensis* into four putative species, leading to a total of 26 putative species (Fig. 4).

Table 1. Species used in phylogenetic analysis of this study.

Species	Locality	Voucher no.	GenBank no.	References
Rhacophorus borneensis	Sabah, Malaysia	BORN 22410	AB781693	Matsui et al. (2013)
Kurixalus idiootocus	Taiwan, China	KUHE 12979	AB933306	Nguyen et al. (2014)
Gracixalus yunnanensis	Bada, Menghai, Yunnan, China	KIZ 20160216	MK234877	Yu et al. (2019)
Gracixalus yunnanensis	Xuelin, Lancang, Yunnan, China	KIZ 20160222	MK234878	Yu et al. (2019)
Gracixalus yunnanensis	Fudong, Lancang, Yunnan, China	KIZ 20160226	MK234880	Yu et al. (2019)
Gracixalus yunnanensis	Fazhanhe, Lancang, Yunnan, China	KIZ 20160228	MK234881	Yu et al. (2019)
Gracixalus sapaensis	Lao Cai, Vietnam	CIB XM-439	GQ285670	Li et al. (2009)
Gracixalus sapaensis	Lao Cai, Vietnam	KUHE 46401	LC011938	Matsui et al. (2015)
Gracixalus sapaensis	Lai Chau, Vietnam	IEBR 2351	EU871425	Nguyen et al. (2008)
Gracixalus sapaensis	Lao Cai, Vietnam	MNHN 1999.5961	AY880503	Matsui et al. (2017)
Gracixalus quangi	Nghe An, Vietnam	AMS R173417	JN862539	Rowley et al. (2011)
Gracixalus supercornutus	Kon Tum, Vietnam	AMS R173887	JN862545	Rowley et al. (2011)
Gracixalus lumarius	Kon Tum, Vietnam	AMS R 176202	KF918412	Rowley et al. (2014)
Gracixalus jinggangensis	Mt. Jinggang, Jiangxi	SYS a003186	KY624587	Zeng et al. (2017)
Gracixalus seesom	Kanchanaburi, Thailand	KUHE 35084	LC011932	Matsui et al. (2015)
Gracixalus quyeti	Cha Noi, Vietnam	VNUH 160706	EU871428	Nguyen et al. (2008)
Gracixalus sp.	Wenshan, Yunnan, China	03320Rao	GQ285669	Li et al. (2009)
Gracixalus gracilipes	Lao Cai, Vietnam	AMS R 177672	KT374014	Rowley et al. (2011)
Gracixalus gracilipes	Pingbian, Yunnan, China	060821196Rao	GQ285668	Li et al. (2009)
Gracixalus ziegleri	Yen Bai, Vietnam	MCC.2018.35	LC642813	Le et al. (2021)
Gracixalus ziegleri	Yen Bai, Vietnam	MCC.2018.15	LC642812	Le et al. (2021)
Gracixalus trieng	Kon Tum Province, Vietnam	AMS R176206	MT328246	Rowley et al. (2020)
Gracixalus trieng	Kon Tum Province, Vietnam	UNS 00342/AMS R176205	MT328245	Rowley et al. (2020)
Gracixalus tianlinensis	Guangxi, China	NHMG 1705016	MH117961	Chen et al. (2018)
Gracixalus tianlinensis	Guangxi, China	NHMG 1705015	MH117960	Chen et al. (2018)
Gracixalus guangdongensis	Hunan, China	CIB HN201108200	LC011936	Matsui et al. (2015)
Gracixalus guangdongensis	Longmen County, Guangdong, China	SYS a004902	MG520193	Wang et al. (2018)
Gracixalus guangdongensis	Nanling Nature Reserve, Guangdong, China	SYS a005750	MG520197	Wang et al. (2018)
Gracixalus guangdongensis	Dawuling Forestry Station, Guangdong, China	SYS a004672	MG520186	Wang et al. (2018)
Gracixalus guangdongensis	Dawuling Forestry Station, Guangdong, China	SYS a004673	MG520187	Wang et al. (2018)
Gracixalus guangdongensis	Dawuling Forestry Station, Guangdong, China	SYS a004685	MG520188	Wang et al. (2018)
Gracixalus guangdongensis	Dawuling Forestry Station, Guangdong, China	SYS a004686	MG520189	Wang et al. (2018)
Gracixalus guangdongensis	Dawuling Forestry Station, Guangdong, China	SYS a004687	MG520190	Wang et al. (2018)
Gracixalus guangdongensis	Dawuling Forestry Station, Guangdong, China	SYS a004699	MG520191	Wang et al. (2018)
Gracixalus guangdongensis	Dawuling Forestry Station, Guangdong, China	SYS a004689	MG520192	Wang et al. (2018)
Gracixalus medogensis	Medog, Xizang, China	KIZ010956	MW023606	Xu et al. (2020)
Gracixalus huaping sp. nov.	Huaping, Guangxi, China	GXNU YU000480	PP083976	This study
Gracixalus huaping sp. nov.	Huaping, Guangxi, China	GXNU YU000511	PP083977	This study
Gracixalus huaping sp. nov.	Huaping, Guangxi, China	GXNU YU000512	PP083978	This study
Gracixalus huaping sp. nov.	Huaping, Guangxi, China	GXNU YU000513	PP083979	This study
Gracixalus huaping sp. nov.	Huaping, Guangxi, China	GXNU YU000514	PP083980	This study
Gracixalus jinxiuensis	Jinxiu, Guangxi, China	KIZ 060821013	EF564524	Yu et al. (2008)
Gracixalus jinxiuensis	Jinxiu, Guangxi, China	KIZ 061210YP	EU215525	Li et al. (2008)
Gracixalus jinxiuensis	Jinxiu, Guangxi, China	SYS a002183	KY624585	Zeng et al. (2017)
Gracixalus jinxiuensis	Jinxiu, Guangxi, China	SYS a002182	KY624584	Zeng et al. (2017)
Gracixalus truongi	Tuan Giao District, Dien Bien Province, Vietnam	IEBR A.5006	OP750514	Tran et al. (2023)
Gracixalus truongi	Tuan Giao District, Dien Bien Province, Vietnam	IEBR A.5005	OP750513	Tran et al. (2023)
Gracixalus nonggangensis	Guangxi, China	NHMG111024	JX841317	Mo et al. (2013)
Gracixalus nonggangensis	Guangxi, China	NHMG20091009	JX841319	Mo et al. (2013)
Gracixalus nonggangensis	Guangxi, China	NHMG200910010	JX841320	Mo et al. (2013)
Gracixalus nonggangensis	Guangxi, China	NHMG1005046	JX841318	Mo et al. (2013)
Gracixalus nonggangensis	Cao Bang, Vietnam	VNMN A.2012.2	JX896684	Nguyen et al. (2013)
Gracixalus nonggangensis	Cao Bang, Vietnam	VNMN A.2012.3	JX896685	Nguyen et al. (2013)
Gracixalus cf. *ananjevae*	Nghe An, Vietnam	VNMN 03012	JN862546	Rowley et al. (2011)
Gracixalus patkaiensis	Kamala Valley beat, Namdapha TR, Changlang dist., Arunachal Pradesh, India	WII-ADA1352	OQ940026	Boruah et al. (2023)
Gracixalus patkaiensis	Kamala Valley beat, Namdapha TR, Changlang dist., Arunachal Pradesh, India	WII-ADA1353	OQ940027	Boruah et al. (2023)

Table 2. Uncorrected *p*-distances (%) between *Gracixalus* species estimated from 16S rRNA sequences. *Gracixalus guangdongensis* and *G. nonggangensis* were divided into different groups as shown in Fig. 2.

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
1	*G. huaping* sp. nov.																										
2	*G. jinxiuensis*	**3.0**																									
3	*G. guangdongensis* (Gg1)	5.6	5.2																								
4	*G. guangdongensis* (Gg2)	6.0	5.8	0.2																							
5	*G. guangdongensis* (Gg3)	5.8	5.6	1.0	0.7																						
6	*G. guangdongensis (Gg4)*	6.5	5.8	2.5	2.2	2.1																					
7	*G. nonggangensis* (Gn1)	8.0	8.0	7.1	7.7	6.9	7.5																				
8	*G. nonggangensis* (Gn2)	8.3	8.0	6.7	7.0	6.5	7.3	2.5																			
9	*G. nonggangensis* (Gn3)	9.1	8.9	6.3	6.2	5.6	6.5	2.7	2.7																		
10	*G.* cf. *ananjevae*	7.4	7.4	4.2	4.6	5.0	5.6	8.8	8.7	8.0																	
11	*G. ziegleri*	5.8	6.0	4.3	4.7	4.3	4.5	7.3	6.8	6.9	5.3																
12	*G. yunnanensis*	6.0	5.8	2.1	2.2	2.3	3.8	7.2	6.5	6.6	4.0	4.0															
13	*G. truongi*	6.7	7.6	4.8	5.3	5.2	5.2	8.6	8.4	8.5	6.5	5.4	4.7														
14	*G. trieng*	5.7	5.5	3.3	3.6	4.0	4.0	6.8	6.6	6.6	5.3	3.7	4.0	4.5													
15	*G. tianlinensis*	6.2	5.8	4.4	4.8	4.2	5.0	7.0	6.3	7.0	6.3	2.4	4.3	5.8	4.0												
16	*G. supercornutus*	9.9	10.9	9.2	9.2	9.2	9.5	12.1	13.2	12.6	11.3	10.6	9.4	9.9	9.4	10.9											
17	*G.* sp. GQ285669	7.9	7.4	5.0	5.5	5.8	6.0	8.9	9.3	8.5	2.3	6.0	5.0	6.7	5.1	6.5	10.9										
18	*G. seesom*	8.3	9.0	8.3	8.3	8.1	8.5	9.7	9.7	9.7	10.2	7.8	8.5	9.9	7.1	7.1	6.5	9.4									
19	*G. sapaensis*	6.5	6.7	4.6	4.3	4.4	5.2	7.1	6.5	6.5	5.3	2.1	4.3	5.5	3.9	2.4	10.7	6.1	8.3								
20	*G. quyeti*	9.2	9.4	9.2	9.4	9.9	8.8	11.4	12.0	11.8	10.4	10.3	9.2	9.8	7.9	9.6	6.1	10.0	7.9	10.1							
21	*G. quangi*	8.4	9.5	7.8	7.5	7.6	7.4	10.7	11.8	11.3	9.8	9.6	8.2	8.8	7.8	9.5	3.2	9.7	5.8	9.3	6.1						
22	*G. patkaiensis*	8.9	9.7	8.8	8.9	8.8	8.6	10.9	11.6	12.0	10.9	9.8	9.7	10.3	8.8	9.2	5.5	10.7	5.1	9.7	7.1	3.8					
23	*G. medogensis*	11.0	10.3	10.4	10.6	10.9	10.4	10.3	10.7	10.5	10.7	9.5	9.7	10.8	9.1	10.1	11.9	10.7	11.1	9.5	12.5	11.9	12.1				
24	*G. lumarius*	15.2	16.0	14.0	14.2	14.2	14.9	16.0	16.0	16.2	14.5	14.5	13.9	15.8	13.9	14.6	15.1	14.6	15.0	14.8	13.8	14.0	13.4	16.2			
25	*G. jinggangensis*	6.3	6.9	5.4	5.3	5.2	5.8	7.5	7.5	7.1	7.3	5.6	5.0	6.5	5.0	6.5	9.2	7.7	9.2	6.1	11.1	7.8	9.6	10.0	14.5		
26	*G. gracilipes*	9.7	10.9	9.7	9.7	9.7	9.5	12.1	12.8	12.8	11.1	10.2	10.5	11.5	9.9	10.3	6.3	11.5	6.0	10.1	6.9	4.8	4.0	11.9	13.6	10.1	

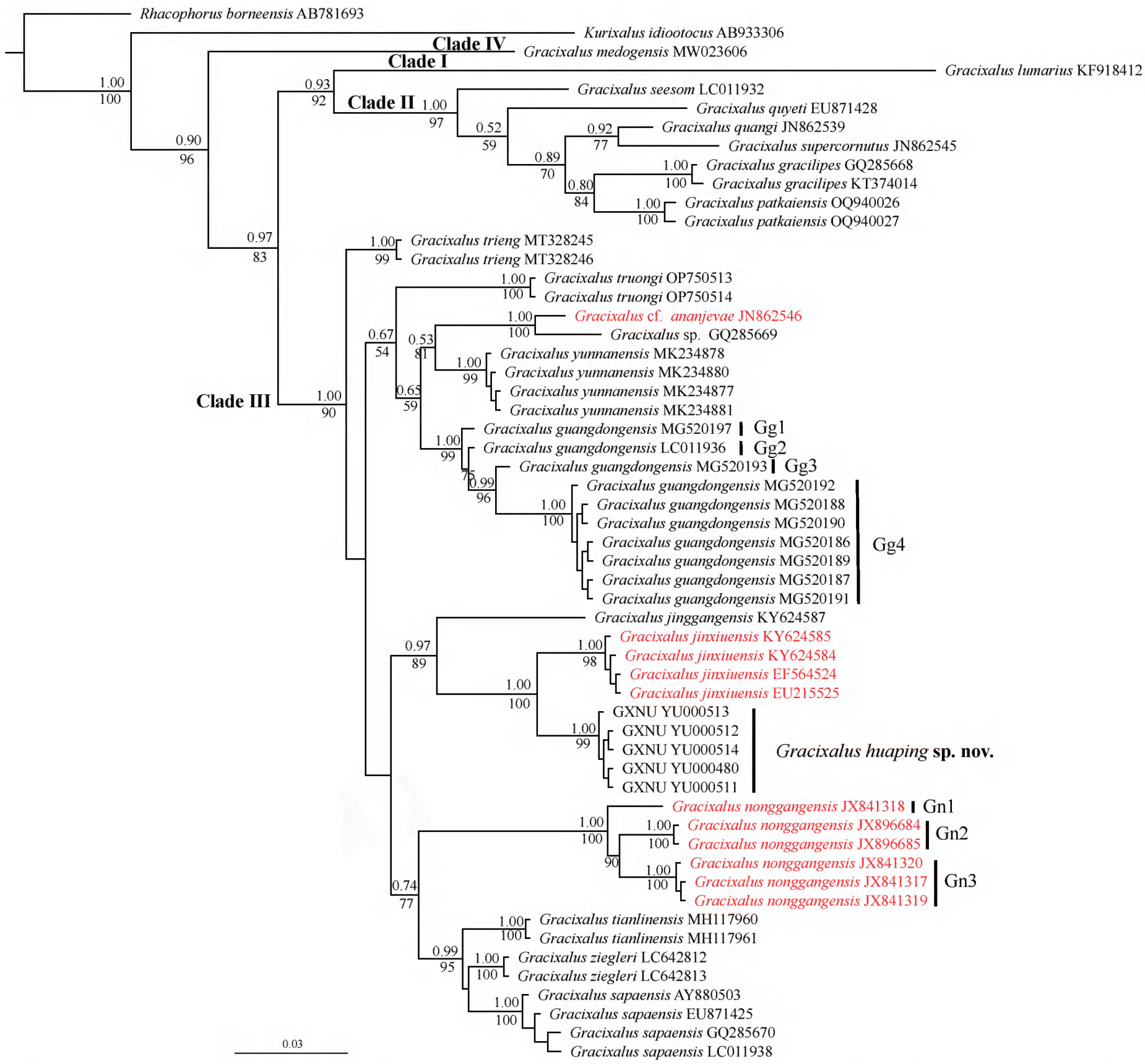

Figure 2. Bayesian phylogram of *Gracixalus* inferred from 547 bp of the 16S rRNA gene. Numbers above and below branches are Bayesian posterior probabilities and ML bootstrap values (only values above 50% are shown), respectively. Species assigned into *Orixalus* by Dubois et al. (2021) are highlighted with red color.

Morphometric analysis

Morphometric data are summarized in Table 3. PCA analysis on Huaping population and *G. jinxiuensis* revealed that the first two principal components accounted for 76.2% of the total variance (Table 4). Dim1 accounted for 54.5% of the total variance, and the loadings of Dim1 were heavily loaded on foot length (FL). The loadings of Dim2 accounted for 21.6% of the total variance and were heavily loaded on tibia length (TL). Differentiation was found along the Dim1 axis between the Huaping population and *G. jinxiuensis* from the type locality (Fig. 5). The results of PCA analysis revealed distinct morphometric differences in foot length between specimens from Huaping and *G. jinxiuensis* from the type locality. Additionally, morphological comparison indicated that specimens from Huaping differ from known species of *Gracixalus* by a series of characters (see below). Therefore, on the basis of the above molecular and morphological evidence, we officially describe the Huaping population as a new species of genus *Gracixalus*.

Taxonomic account

***Gracixalus huaping* sp. nov.**

https://zoobank.org/2627F9B4-6B7B-4D17-B727-790A6CBC2F77

Figs 6A, 7, 8

Type material. ***Holotype*** • GXNU YU000517, an adult male, was collected on 3 May 2022 by Guohua Yu from Huaping National Natural Reserve, Longsheng, Guangxi, China (25°36'44"N, 109°56'19"E, 1474 m elevation).

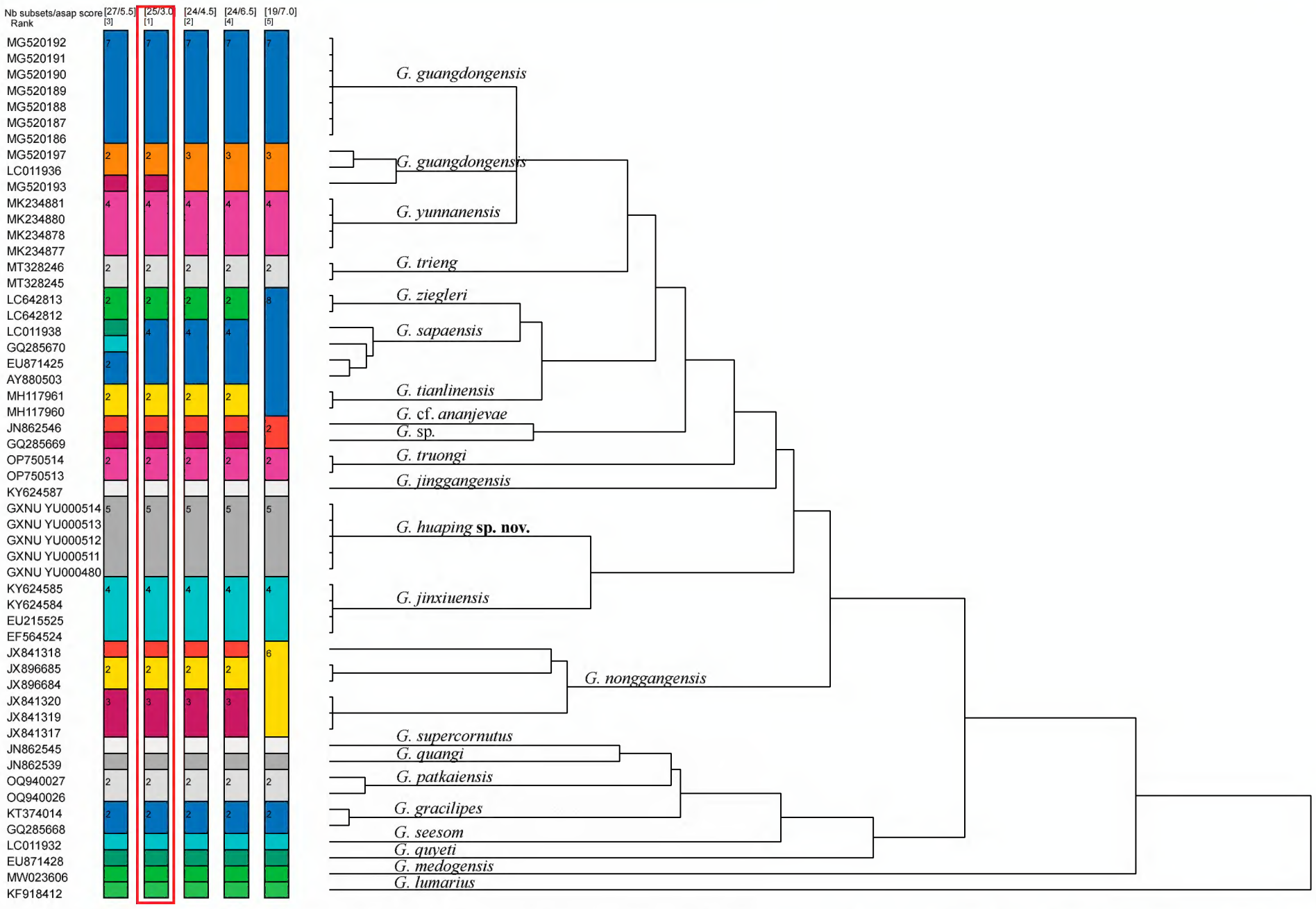

Figure 3. ASAP species delimitation in genus *Gracixalus* based on 16S sequences. The best partition with the lowest score is highlighted with a red frame.

Table 3. Measurements (mm) of *Gracixalus huaping* sp. nov. from the type locality and *G. jinxiuensis* from Jinxiu. Data of 63A9033 came from Hu et al. (1978). Abbreviations defined in text. * Holotype.

Character	*Gracixalus huaping* sp. nov.								*Gracixalus jinxiuensis*			
	GXNU YU000512	GXNU YU000516	*GXNU YU000517	GXNU YU000511	GXNU YU000513	GXNU YU000514	GXNU YU000515	63A9033	GXNU YU000812	GXNU YU000813	GXNU YU000486	GXNU YU000858
Sex	M	M	M	F	F	F	F	M	M	M	M	M
SVL	26.6	28.8	28.6	32.8	31.9	29.8	31.4	23.5	30.5	30.7	18.8	34.3
HL	8.2	9.0	9.0	10.9	9.4	9.3	9.5	9.0	11.6	11.7	7.2	13.0
HW	10.3	10.8	10.9	12.4	11.4	11.0	11.8	9.2	11.2	11.3	7.7	12.9
IND	3.0	3.4	3.3	3.6	3.4	3.2	3.4	3.0	3.9	4.0	2.7	4.4
IOD	3.2	3.4	3.3	3.7	3.4	3.4	3.8	2.9	3.5	4.1	2.5	4.0
ED	3.2	3.3	3.2	3.6	3.5	3.3	3.6	3.0	4.0	4.6	3.0	4.2
TD	1.5	1.6	1.5	1.8	1.9	1.7	1.7	1.2	2.1	2.0	1.3	2.1
FHL	13.6	14.7	14.3	16.4	15.7	16.1	15.9	11.5	14.5	14.5	9.3	16.0
TL	13.2	14.7	14.2	15.2	14.9	15.5	15.7	11.7	14.1	14.9	9.5	16.9
TFL	18.6	20.4	19.4	22.1	21.8	22.4	21.8	16.0	19.5	20.7	11.9	23.1
FL	11.8	13.0	12.4	14.1	14.0	14.5	14.1	10.0	12.4	12.7	7.4	14.3
UEW	2.5	2.5	2.7	3.2	2.9	2.6	3.0	2.4	3.1	3.1	1.9	3.4
SL	3.0	3.2	3.2	3.8	3.6	3.5	3.7	3.7	4.7	4.8	2.9	5.1

Paratypes • Two adult males (GXNU YU000512, GXNU YU000516) and four adult females (GXNU YU000511, GXNU YU000513, GXNU YU000514, GXNU YU000515) were collected from the type locality at the same time by Guohua Yu.

Etymology. The specific epithet is named for Huaping, Guangxi, China, where the species was collected. We suggested "Huaping small tree frog" for the common English name and "花坪纤树蛙 (Huā Píng Xiān Shù Wā)" for the common Chinese name.

Diagnosis. The new species is assigned to genus *Gracixalus* based upon the following set of morphological characters: the presence of intercalary cartilage between terminal and penultimate phalanges of digits, tips of digits enlarged to discs bearing circum-marginal grooves, vomerine teeth absent, inner (first and second)

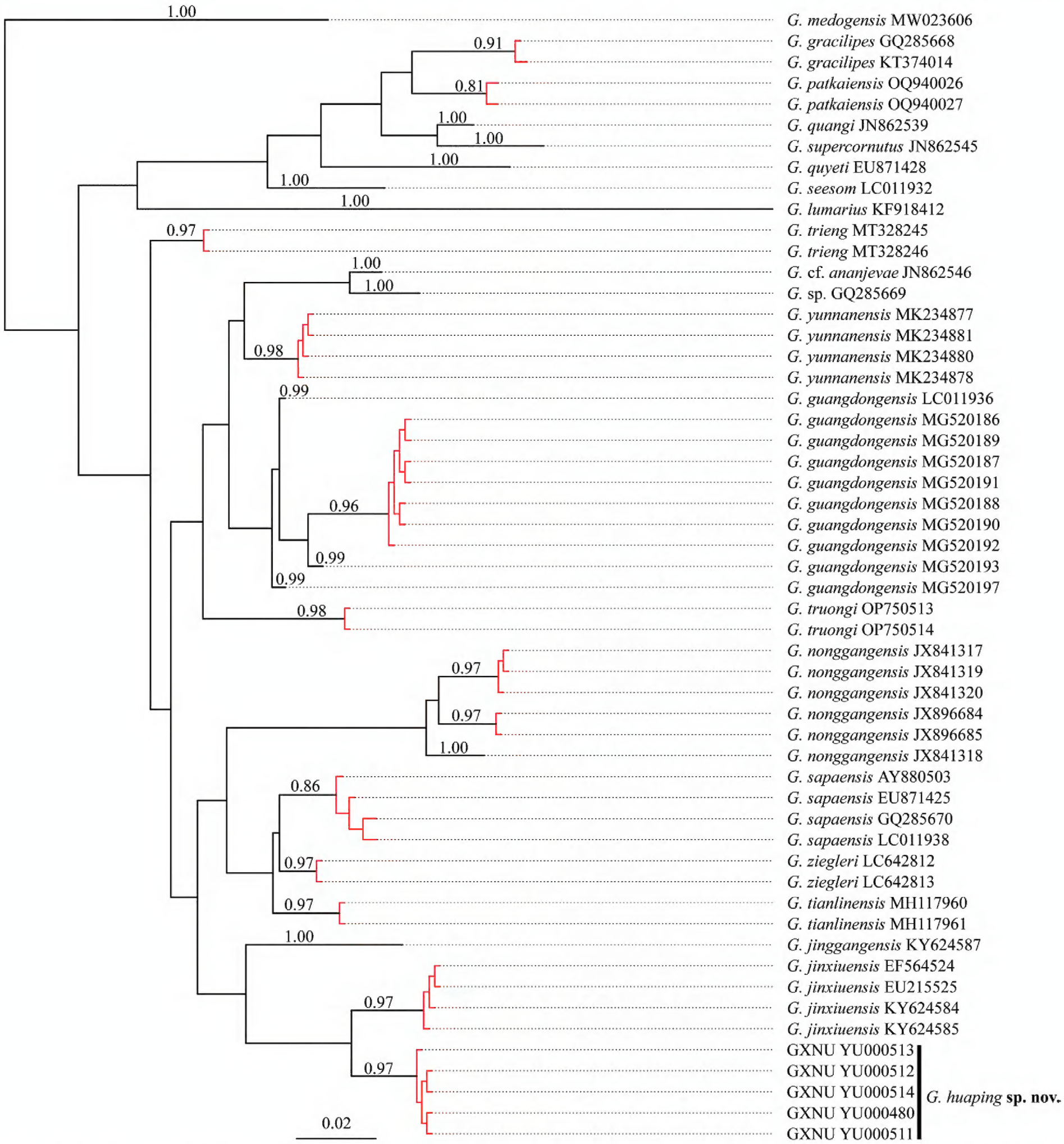

Figure 4. The bPTP species delimitation based on the Bayesian phylogenetic tree inferred from 16S rRNA sequences. The Bayesian support values above branches indicate the posterior probabilities of those taxa form one species.

and outer (third and fourth) fingers not opposable, and an inversed Y-shaped dark brown marking on the dorsum (Fei 1999; Yu et al. 2019; Tran et al. 2023).

The new species can be distinguished from its congeners by a combination of the following morphological characters. (1) SVL 26.6–28.8 mm in males and 29.8–32.8 mm in females; (2) vomerine teeth absent; (3) dorsal surface yellowish brown; (4) few conical tubercles on dorsum; (5) internal vocal sac in males, vocal sac opening oval and rim of the opening dark brown; (6) throat smooth; (7) finger webbing rudimentary; (8) linea masculina present in males; (9) snout rounded; (10) tibiotarsal articulation reaching eye; (11) tibiotarsal projection absent; (12) ventral surface translucent, yellowish-white; (13) belly granular; (14) nuptial pads present on finger I; (15) heels overlapping when legs at right angle to body; (16) iris golden brown; and (17) flank rough with white tubercles.

Description of holotype. Small size (SVL 28.6 mm; Table 3); head wider (HW 10.9 mm) than long (HL 9.0 mm); snout rounded, slightly projecting beyond margin of lower jaw in ventral view, rounded in profile; canthus rostralis rounded; nostril oval, protuberant, closer

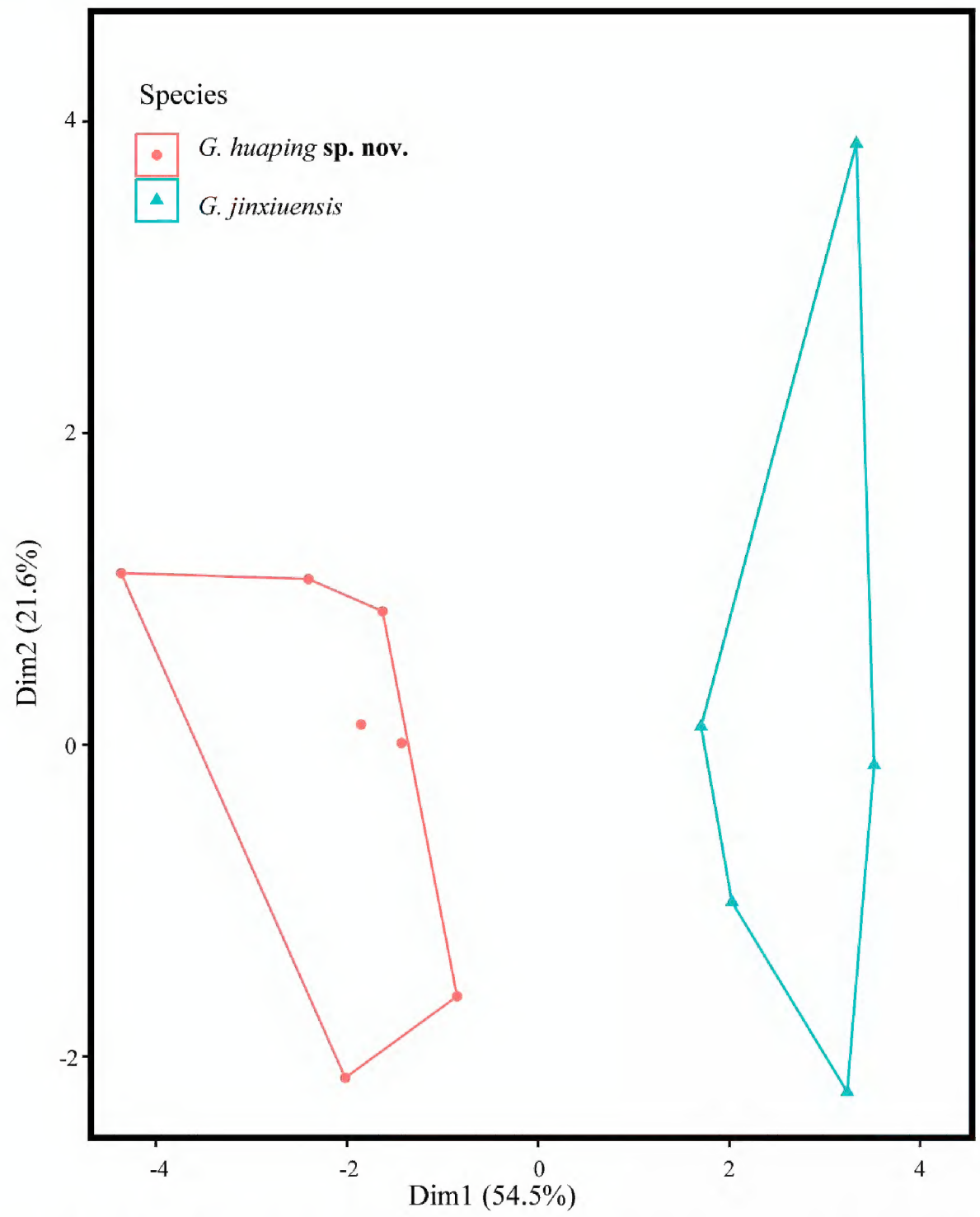

Figure 5. Scatterplot of principal components 1 and 2 of size-adjusted morphometric data of *Gracixalus huaping* sp. nov. and *G. jinxiuensis*.

Table 4. Factor loadings of the first two principal components of 12 size-adjusted species morphometric characteristics of *Gracixalus huaping* sp. nov. and *G. jinxiuensis*.

Character	Dim1	Dim2
Variance	6.542	2.597
% of var.	54.519	21.639
Cumulative % of var.	54.519	76.158
HL	0.850	-0.172
HW	0.304	0.771
IND	0.890	0.398
IOD	0.594	0.663
ED	0.835	0.466
TD	0.668	0.075
FHL	-0.843	0.405
TL	-0.342	**0.790**
TFL	-0.838	0.076
FL	**-0.927**	0.011
UEW	0.701	-0.509
SL	0.747	-0.313

to tip of snout than eye; loreal region oblique, slightly concave; interorbital distance (IOD 3.3 mm) equal to internarial distance (IND 3.3 mm) and greater than upper eyelid width (UEW 2.5 mm); eye large, horizontal diameter (ED 3.2 mm) equal to snout length (SL 3.2 mm); pupil oval, horizontal; tympanum distinct (TD 1.5 mm), nearly equal to half of ED; supratympanic fold distinct, extending from posterior corner of eye to above insertion of arm; vomerine teeth absent; tongue notched posteriorly; single internal vocal sac, vocal sac opening oval and rim of the opening dark brown.

Forelimbs relatively strong; forearm and hand length (FHL 14.3 mm) 50% of SVL; relative finger lengths I < II < IV < III; fingers webbing rudimentary; tips of all fingers expanded into discs with circum-marginal grooves; subarticular tubercles prominent and rounded, formula 1, 1, 2, 2; two metacarpal tubercles, the inner oval and the outer rounded; nuptial pad present at the base of the first finger, prominent.

Heels overlapping when legs at right angle to body; the relative lengths of the toes I < II < III = V < IV; tibiotarsal articulation reaching the middle of the eye when hindlimb adpressed to body; tip of toes expanded into discs with circum-marginal grooves; toe discs slightly smaller than finger discs; toes webbed, webbing formula I2–1.5II1.3–2III1.2–2IV2–1.3V; subarticular tubercles

Figure 6. Lateral, dorsal, and ventral views of the holotype of *Gracixalus huaping* sp. nov. (**A.** voucher no. GXNU YU000517) and topotype of *G. jinxiuensis* (**B.** GXNU YU000813) in life. The linea masculina was highlighted with arrows. Photos by Guohua Yu.

prominent, round, formula 1, 1, 2, 3, 2; supernumerary tubercles present; inner metatarsal tubercle oval, outer metatarsal tubercle absent.

Dorsal surface rough, scattered with a few conical tubercles on the upper eyelids, head, dorsum, and limbs; flank and head side rough, scattered with white tubercles; throat relatively smooth; chest, belly, and ventral surface of thighs rough with many white tubercles.

Coloration of holotype. In life, dorsal surface light yellowish-brown; a dark yellow-brown inverted Y-shaped marking on dorsal surface covering interorbital region, bifurcating into two branches on shoulder and extending posteriorly; dorsal surface of limbs yellow-brown with dark brown stripes; flanks and ventral yellow white; iris golden brown (Fig. 6A).

In preservative, color faded but pattern same as in life; dorsal surface gray-brown with a dark brown inverted Y-shaped marking; light brown stripes on limbs; ventral surface gray-white (Fig. 7).

Sexual dimorphism. Males have nuptial pad on the base of finger I and internal single subgular vocal sac. Additionally, males have linea masculina.

Morphological variation. The inverted Y-shaped marking on the dorsum in paratype GXNU YU000513 is discontinuous. Paratypes GXNU YU000512, GXNU YU000515, and GXNU YU000516 have much more dark blotches on throat, chest, and belly (Fig. 8).

Distribution and ecology. The new species is known only from the type locality. Probably the new species is restricted to bamboo forest with evergreen forest (Fig. 9a). All specimens were collected in daylight and were found in open-ended bamboos filled with a little water. Eggs with gel nests were deposited on the walls of open-ended bamboos (Fig. 9b), implying that the new species has entered breeding season in April. In daylight no calls were heard.

Comparisons. *Gracixalus huaping* sp. nov. differs from its sister taxon *G. jinxiuensis* by having smaller maximum body size in males (SVL up to 28.8 mm vs. up to 34.3 mm), vocal sac opening oval and rim of the opening dark brown (vs. vocal sac opening slitted and edge of the opening immaculate; Fig. 10), skin of venter more granular (Fig. 10), linea masculina present (vs. absent; Fig. 6), longer foot, and heels overlapped (vs. just meeting) (Table 5).

The new species differs from *G. ananjevae* by having smaller body size (SVL 26.6–28.8 mm in males and 29.8–32.8 mm in females vs. male 32 mm and female 43 mm), finger webbing rudimentary (vs. finger webbing formula I2–2II2–basalIII3–2.75IV), and presence of white granules around anus and on limbs (vs. absent); from *G. carinensis* by having smaller body size (SVL 26.6–28.8 mm in males and 29.8–32.8 mm in females vs. 30.2–38.1 mm in males and 40.3–41.8 mm in females), less developed toe webbing, and conical tubercles on dorsum present (vs. absent); from *G. gracilipes* by having bigger body size in males (SVL 26.6–28.8 mm vs. 20–24 mm), snout rounded (vs. triangularly pointed), dorsal surface light yellowish-brown (vs. greenish), and nuptial pads present just on finger I (vs. nuptial pads present on

Figure 7. Views of the holotype of *Gracixalus huaping* sp. nov. (voucher no. GXNU YU000517) in preservative. Photos by Guohua Yu.

fingers I and II); and from *G. guangdongensis* by skin of throat smooth (vs. granular), white patch on temporal region absent (vs. present), dorsal surface light yellowish-brown (vs. brown), ventral surface translucent and yellowish-white, throat smooth, chest and belly granular (vs. throat and chest creamy white, belly light brown, semi-transparent), and iris golden-brown (vs. brown).

Gracixalus huaping sp. nov. differs from *G. jinggangensis* Zeng, Zhao, Chen, Chen, Zhang & Wang, 2017 by nuptial pads present just on finger I (vs. nuptial pads present on fingers I and II) and heels overlapped (vs. just meeting); from *G. lumarius* by having smaller body size (SVL 26.6–28.8 mm in males and 29.8–32.8 mm in females vs. 38.9–41.6 mm in males and 36.3 mm in a female), dorsal surface rough scattered with tubercles (vs. dorsal surface of head and back covered in dense and distinctive white conical tubercles), dorsum light yellowish-brown (vs. yellow), and supratympanic fold distinct (vs. indistinct); and from *G. medogensis* by dorsal surface and flank rough scattered with tubercles (vs. relatively smooth), dark brown mark beginning between eyes bifurcated into two wide branches after the back of shoulders (vs. bifurcated into two relatively narrow branches before the back of shoulders), dorsal color light yellowish-brown (vs. grass green), finger webbing rudimentary (vs. absent), and ventral surface yellowish-white (vs. pale green).

Gracixalus huaping **sp. nov.** differs from *G. nonggangensis* by having smaller body size (SVL 26.6–28.8 mm in males and 29.8–32.8 mm in females vs. 29.9–35.3 mm in males and 33.6–38.0 mm in females), dorsal surface light yellowish-brown with dark-brown mark (vs. yellowish-olive with dark-green mark), finger webbing rudimentary (vs. absent), linea masculina present (vs. absent), nuptial pads on finger I (vs. absent), and iris golden-brown (vs. olive); from *G. quangi* by having larger body size (SVL 26.6–28.8 mm in males and 29.8–32.8 mm in females vs. 21.4–24.5 mm in males and 26.6–27.3 mm in females), dorsal surface light yellowish-brown (vs. olive-green), vocal sac internal (vs. external), finger webbing rudimentary (vs. absent), snout rounded (vs. triangularly pointed), brown under the supratympanic fold (vs. pale turquoise), tibiotarsal projection absent (vs. present), ventral surface translucent and yellowish-white with dark blotches (vs. opaque white with translucent pale-green margins), and iris golden-brown (vs. bronze); and from *G. quyeti* by dorsal color light yellowish-brown (vs. brownish to moss-green), head wider than long (vs. head longer than wide), tibiotarsal articulation reaching eye (vs.

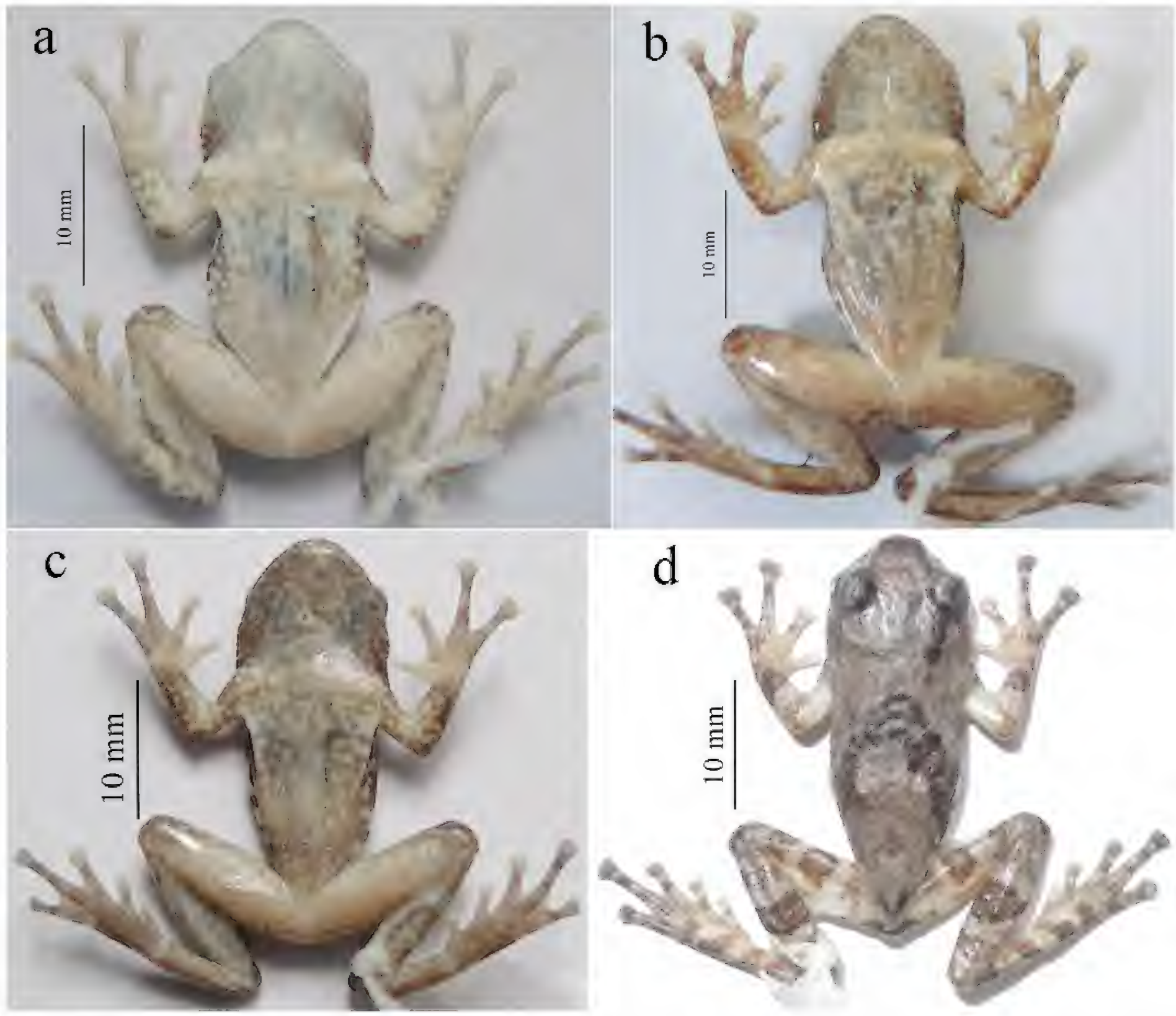

Figure 8. Ventral views of paratypes GXNU YU000512 (**a**), GXNU YU000515 (**b**), and GXNU YU000516 (**c**) in preservative and a dorsal view of paratype GXNU YU000513 (**d**). Photos by Guohua Yu.

reaching to snout), supratympanic fold distinct (vs. indistinct), throat white, clouded with dark in preservative (vs. yellow-white with brown marbling), and forelimb, dorsal parts of thighs, tibia, and foot brown with dark brown bands (vs. moss-green with dark brown bands).

The new species differs from *G. sapaensis* Matsui, Ohler, Eto & Nguyen, 2017 by having tubercles on dorsum (vs. dorsum smooth), white warts on edge of upper jaw, temporal region, flanks and limbs (vs. absent), throat, chest, belly and ventral surface of forelimbs white with dark specks (vs. light yellow), and dorsal surface light yellowish-brown (vs. golden ochre); from *G. seesom* by having bigger body size (SVL 26.6–28.8 mm in males and 29.8–32.8 mm in females vs. 21.6–23.0 mm in males and 23.2–25.4 mm in females), snout rounded (vs. triangularly pointed), dorsal surface rough scattered with tubercles (vs. smooth), dorsal surface light yellowish-brown with dark brown inverted Y-shaped marking (vs. tan), and nuptial pads on finger I (vs. absent); from *G. supercornutus* by having bigger body size (SVL 26.6–28.8 mm in males vs. 22.0–24.1 mm), dorsal surface light yellowish-brown (vs. dorsum green with brown spots), snout rounded (vs. pointed), white patch on temporal region absent (vs. present), tubercles on dorsal surface small (vs. considerable bigger horn-like projections in supraorbital area, around cloaca, and on dorsum, forelimbs and hindlimbs), and tibiotarsal projection absent (vs. present); and from *G. tianlinensis* by having smaller body size (SVL 26.6–28.8 mm in males and 29.8–32.8 mm in females vs. 30.3–35.9 mm in males and 35.6–38.7 mm in females), vocal sac internal (vs. external), finger webbing rudimentary (vs. absent), and nuptial pads only on finger I (vs. on fingers I and II), and iris golden-brown (vs. bronze).

Gracixalus huaping sp. nov. differs from *G. trieng* Rowley, Le, Hoang, Cao & Dau, 2020 by having smaller body size in males (SVL 26.6–28.8 mm vs. 37.2–41.4 mm), throat and chest white with dark specks (vs. throat and chest yellow or yellowish brown with pinkish mottling), linea masculina present (vs. absent), and nuptial pads only on finger I (vs. on fingers I and II); from

Figure 9. Habitat of *Gracixalus huaping* sp. nov. at the type locality (**a**) and eggs of the new species found in open-ended bamboo. Photos by Guohua Yu.

G. yunnanensis by vocal sac internal (vs. external), and flank rough with tubercles (vs. smooth); from *G. ziegleri* Le, Do, Tran, Nguyen, Orlov, Ninh & Nguyen, 2021 by skin of throat smooth (vs. granular), linea masculina present (vs. absent), and tibiotarsal articulation reaching eye (vs. reaching tip of snout); and from *G. truongi* Tran, Pham, Le, Nguyen, Ziegler & Pham, 2023 by having smaller body size (SVL 26.6–28.8 mm in males and 29.8–32.8 mm in females vs. 32.2–33.1 mm in males and 37.6–39.3 mm in females), dorsal surface light yellowish-brown (vs. moss green with grey), dorsal surface rough scattered with sparse tubercles (vs. smooth), and linea masculina present (vs. absent).

Discussion

Gracixalus jinxiuensis was originally described from Jinxiu, Guangxi, China, and once recorded widely in China, Vietnam, and Laos (Orlov et al. 2004; Nguyen et al. 2008; Rowley et al. 2011; Shen 2014; Matsui et al. 2015, 2017; Tran et al. 2023). However, recent studies found that misidentifications were involved within previous records of *G. jinxiuensis*, and distribution of this species is probably restricted to Guangxi, China (Ziegler et al. 2014; Yu et al. 2019). In Guangxi, *G. jinxiuensis* was recorded in Xing'an, Guanyang, Ziyuan, Longsheng, Longzhou, and Jinxiu counties (Mo et al. 2014). In this study, we found that the Huaping population represents a distinct species, implying that the taxonomic status of other records of *G. jinxiuensis* also needs further examination.

The discovery of *G. huaping* sp. nov. brings the number of species in the genus *Gracixalus* to 21. Based on the results of phylogenetic analyses and species delimitation, we consider that species diversity of *Gracixalus* remains underestimated. The specimen 03320Rao (GQ285669) was collected from Wenshan, Yunnan, China, and considered conspecific to the specimen (VNMN 03012) under the name *G.* cf. *ananjevae* (Nguyen et al. 2020). The genetic divergence between them is 2.3%, which is nearly equal to the divergence between *G. ziegleri* and *G. tianlinensis* (2.4%) and is in the range of divergence between *G. guangdongensis* and *G. yunnanensis* (2.1% to 3.8%), and our analyses of species delimitation assigned them into two putative species. Therefore, the Wenshan population may represent a cryptic species pending further morphological evidence. *Gracixalus guangdongensis* is known from the type locality (Dawuling Forestry Station, Yunkaishan National Nature Reserve, Guangdong), Nanling National Nature Reserve, Nankunshan

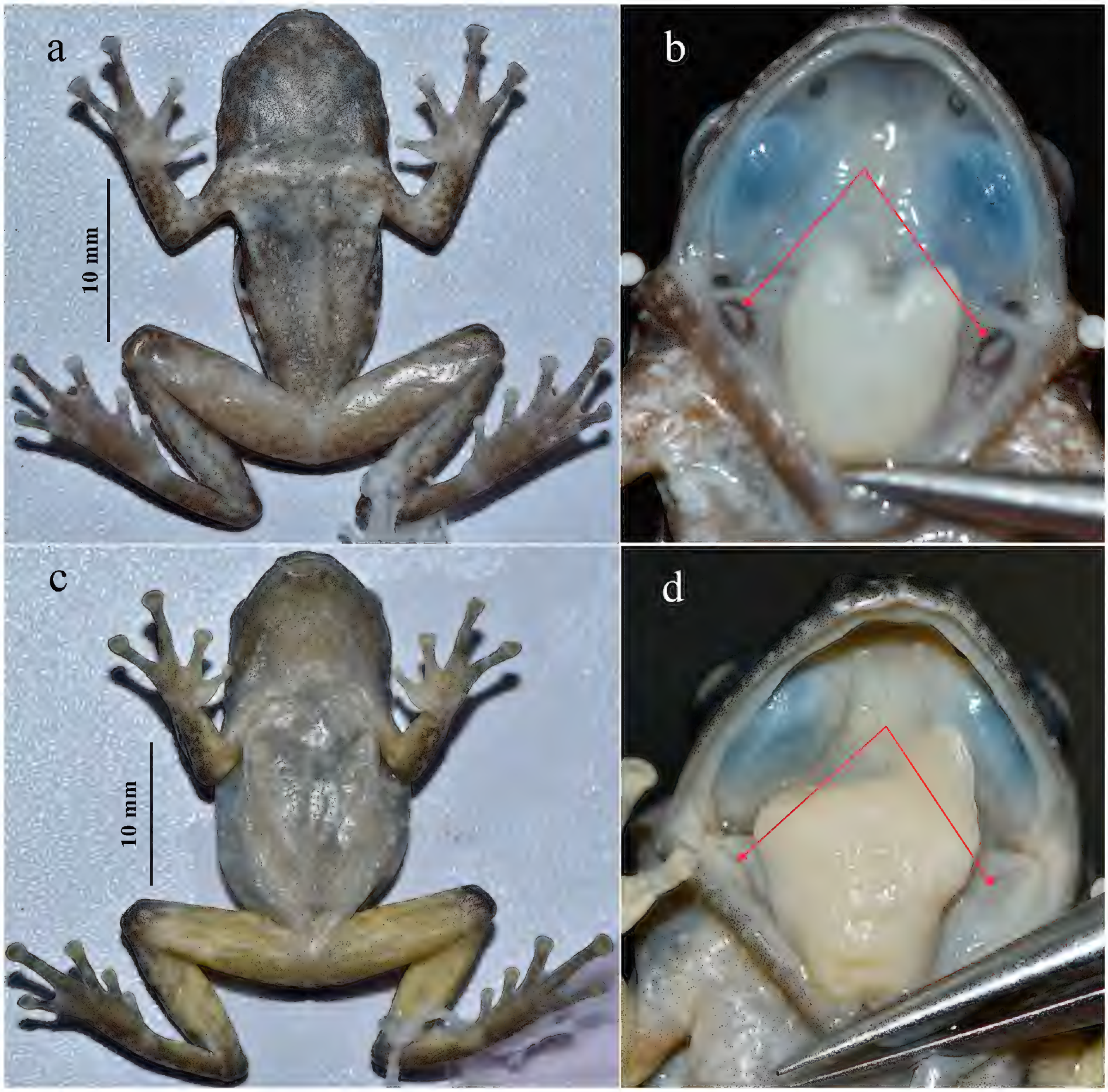

Figure 10. Ventral views and vocal sac openings of *G. huaping* sp. nov. (GXNU YU000516; **a, b**) and topotype of *G. jinxiuensis* (GXNU YU000813; **c, d**). Photos by Guohua Yu.

National Nature Reserve, and Mangshan National Nature Reserve (AmphibiaChina 2024). In this study, the samples from these four sites were assigned into three or four putative species, one of which only consisted of the samples from the type locality. Morphologically, according to the original description of *G. guangdongensis* (Wang et al. 2018), specimens from Nankunshan have smaller body size (26.4–28.0 mm in males) than specimens from the type locality (SVL 28.7–34.7 mm in males) and Nanling (28.5–33.1 mm in males). These remind us that probably additional studies on the taxonomy of *G. guangdongensis* based on more morphological and molecular data are needed. In addition, we revealed that *G. nonggangensis* is comprised of three lineages, and they were grouped into three putative species by both ASAP and bPTP analyses. The lineage consisting of specimens VNMN A.2012.2 (JX896684) and VNMN A.2012.3 (JX896685) was originally described as *G. waza* by Nguyen et al. (2013) but was synonymized with *G. nonggangensis* by Nguyen et al. (2020). Morphologically, males of *G. waza* have developed nuptial pads on finger I (Nguyen et al. 2013), and males of *G. nonggangensis* lack nuptial pads (Mo et al. 2013). The genetic divergences between these three lineages ranged from 2.5% to 2.7%, being in the range of divergence between *G. yunnanensis* and *G. guangdongensis* (2.1% to 3.8%) and greater than the divergence between *G. ziegleri* and *G. tianlinensis* (2.4%). Therefore, we agree with Le et al. (2021) that more data are needed to investigate the taxonomy of *G. nonggangensis* and *G. waza*.

Table 5. Morphological characters for comparisons among *Gracixalus* species. "?" = not known or not clearly defined in the literature.

Species	SVL in males (mm)	Conical tubercles on dorsum	Dorsal color in life	Vocal sac	Skin of body	Skin of throat	webbing between fingers	Linea masculina	Tibiotarsal articulation	Snout	White patch on temporal region	Tibiotarsal projection	Supratym-panicfold	ventral	Nuptial pads	Heels	Iris	References
G. huaping sp. nov.	26.6–28.8	present	light yellowish–brown	Internal, vocal sac opening oval and rim the opening brown	rough with sparsely scattered with tubercles	smooth	rudimentary	present	reaching eye	rounded	absent	absent	distinct	venter surface translucent and yellowish-white, throat smooth, chest and belly granular	on finger I	overlapping	golden brown	This study
G. jinxiuensis	23.5–34.3	present	brown	Internal, vocal sac opening slitted and edge of the opening immaculate	rough with tubercles	granular	rudimentary	absent	reaching eye	rounded	absent	absent	distinct	gray-brown with dark marbling	on fingers I	just meeting	dark brown	Zeng et al. (2017) and This study
G. ananjevae	32.0	absent	?	?	coarsely granular	plain	rudimentary	?	reaching eye	slightly pointed	absent	absent	distinct	immaculate	on finger I	overlapping	?	Rowley et al. (2011)
G. carinensis	30.2–38.1	absent	purplish, reddish, or grayish brown	internal	?	granular	rudimentary	?	reaching eye	rounded	absent	absent	distinct	immaculate white	?	?	?	Yu et al. (2019)
G. gracilipes	20.0–24.0	absent	greenish	internal	smooth with white stripe	smooth	rudimentary	?	reaching eye	triangularly pointed	present	absent	distinct	yellowish white	on fingers I and II	overlapping	brown	Rowley et al. (2011)
G. guangdongensis	26.1–34.7	present	brown	?	rough, black blotches	granular	absent	?	reaching between eye and nostril	triangularly pointed	absent	present	distinct	throat and chest creamy white, belly light brown, semi-transparent	on finger I	overlapping	brown	Wang et al. (2018)
G. jinggangensis	27.9–33.8	absent	brown to beige	?	rough with tubercles	granular	rudimentary	?	reaching eye	triangularly pointed	absent	absent	distinct	Throat and chest dirty white with dark specks, belly white anteriorly with dark marking and posteriorly yellowish, semi-transparent	on fingers I and II	just meeting	golden	Zeng et al. (2017)
G. lumrius	38.9–41.6	present	yellow	external	?	granular	rudimentary	?	?	rounded	absent	absent	indistinct	opaque pink	on fingers I	?	dark gold	Rowley et al. (2014)

Species	SVL in males (mm)	Conical tubercles on dorsum	Dorsal color in life	Vocal sac	Skin of body	Skin of throat	webbing between fingers	Linea masculina	Tibiotarsal articulation	Snout	White patch on temporal region	Tibiotarsal projection	Supratym-panicfold	ventral	Nuptial pads	Heels	Iris	References
G. medogensis	26.5	absent	grass green	internal	?	granular	absent	present	reaching eye	rounded	absent	absent	distinct	pale green	on fingers I	overlapping	?	Xu et al. (2020)
G. nonggangensis	29.9–35.3	absent	yellowish–olive with dark–green mark	internal	rough with tubercles	granular	absent	absent	reaching tip of snout	rounded	absent	absent	distinct	white with dark marbling, semi-transparent	absent	overlapping	olive	Mo et al. (2013)
G. quangi	<25.0	present, small	olive–green	external	with black blotches	smooth	absent	?	?	triangularly pointed	present	present	distinct	opaque white with translucent pale green margins	on fingers I	?	bronze	Rowley et al. (2011)
G. quyeti	?	present	brownish to moss–green	?	rough with sharp tubercles	smooth	rudimentary	?	reaching to snout	rounded	absent	absent	indistinct	belly immaculate white	?	overlapping	?	Nguyen et al. (2008)
G. sapaensis	21.0–37.0	absent	golden ochre	?	coarsely scattered with large tubercles	?	rudimentary	?	reaching eye	rounded	absent	absent	distinct	throat, chest, and belly light yellow, with dark marking	on finger I	overlapping	golden	Matsui et al. (2017)
G. seesom	21.6–23.0	absent	tan	?	with large tubercles and white blotches	smooth	rudimentary	?	reaching between eye and nostril	triangularly pointed	absent	absent	distinct	anterior belly opaque white and posterior belly translucent	absent	overlapping	golden	Matsui et al. (2015)
G. supercornutus	22.0–24.1	present, bigger horn-like	brown spots green with	?	?	granular	?	?	?	pointed	present	present	distinct	light with white spots	?	?	?	Rowley et al. (2011)
G. tianlinensis	30.3–35.9	absent	brown to beige	external	?	granular	absent	?	?	rounded	absent	absent	distinct	throat and chest gray with dark specks, belly creamy white, opaque	on fingers I and II	?	bronze	Chen et al. (2018)
G. yunnanensis	26.0–34.2	present, small	yellow brown or red brown	external	smooth, no black blotches	granular	rudimentary	present	reaching eye	rounded	absent	absent	distinct	orangish with yellow spots, immaculate, semi–transparent	on finger I	overlapping	bronze	Yu et al. (2019)
G. trieng	37.2–41.4	present	brown or yellowish	present	?	granular	rudimentary	?	?	rounded	?	absent	distinct	throat and chest mostly yellowish brown, with dark mottling; belly pinkish brown	on fingers I and II	?	pale gold	Rowley et al. (2020)

Species	SVL in males (mm)	Conical tubercles on dorsum	Dorsal color in life	Vocal sac	Skin of body	Skin of throat	webbing between fingers	Linea masculina	Tibiotarsal articulation	Snout	White patch on temporal region	Tibiotarsal projection	Supratym-panicfold	ventral	Nuptial pads	Heels	Iris	References
G. ziegleri	28.1–30.5	present	yellowish brown	internal	rough, black blotches	granular	rudimentary	absent	reaching tip of snout	triangularly pointed	absent	absent	distinct	throat and chest dirty white with moderate dark specks; belly white cream with large dark blotches, translucent posteriorly and dark yellowish	on finger I	overlapping	golden	Le et al. (2021)
G. truongi	32.2–33.1	absent	moss green with grey	internal	smooth	smooth	absent	absent	reaching between eye and nostril	rounded	absent	absent	distinct	immaculate white	on finger I	overlapping	brown and moss green	Tran et al. (2023)
G. patkaiensis	23.6–26.5	absent	chartreuse green	external	with spinules of different size	smooth	absent	?	reaching tip of snout	pointed	present	absent	distinct	belly granular, below skin semi-transparent, light green with large white patch covering, middle of throat	on finger I	?	golden yellow	Boruah et al. (2023)

Previous studies revealed three major clades (clades I–III; Fig. 2) in the genus *Gracixalus* (Chen et al. 2018; Yu et al. 2019; Tran et al. 2023; Boruah et al. 2023). In this study, we further recovered *G. medogensis* as a major clade (clade IV), and it is sister to the clade consisting of all other *Gracixalus* species. Recently, based on a phylogenetic analysis of limited sampling of *Gracixalus*, Dubois et al. (2021) coined a new genus, *Orixalus*, to accommodate *G. ananjevae*, *G. carinensis*, *G. jinxiuensis*, *G. nonggangensis*, and *G. waza* and retained other species in genus *Gracixalus*. However, this taxonomic arrangement is not compatible with the phylogenetic framework of *Gracixalus* revealed by this and previous studies (e.g., Chen et al. 2018; Yu et al. 2019; Tran et al. 2023; Boruah et al. 2023), rendering both *Orixalus* and *Gracixalus* sensu stricto non-monophyletic. Moreover, morphologically *Orixalus* and *Gracixalus* sensu stricto cannot be distinguished effectively based on the diagnosis provided by Dubois et al. (2021). Therefore, we agree with Mahony et al. (2024) that the division of *Gracixalus* into two genera suggested by Dubois et al. (2021) is not valid and *Orixalus* is a junior subjective synonym of *Gracixalus*.

The key to the five species of *Gracixalus* in Guangxi, China

1 Upper eyelid dark brown with numerous tubercles; snout triangularly pointed; white blotch on temporal region present ..... *G. gracilipes*
– Upper eyelid no dark brown with numerous tubercles; snout rounded; no white blotch on temporal region 2
2 Vocal sac is external ..... *G. tianlinensis*
– Vocal sac is internal 3
3 Dorsal surface green ..... *G. nonggangensis*
– Dorsal surface brown 4
4 Vocal sac opening slitted and edge of the opening immaculate; linea masculina absent ..... *G. jinxiuensis*
– Vocal sac opening oval and rim of the opening dark brown; linea masculina present ..... *G. huaping* sp. nov.

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